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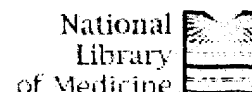
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## [Purification and some properties of extracellular beta-glucosidase from *Rhizopus japonicus* IFO5318]

[Article in Chinese]

**Chen X, Fujio Y.**

College of Life Sciences, Wuhan University, Wuhan 430072.

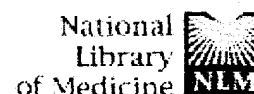
The beta-glucosidase from *Rhizopus japonicus* IFO5318 was purified by Ammonium sulfate salting out and column chromatographies with the recovery of 22%. The molecular weight of the enzyme was about  $4.0 \times 10^5$ , consisting of four identical subunits; The optimum reaction temperature and pH for the beta-glucosidase were 55 degrees C and pH 5.5, respectively; While the enzyme was sensitive to heat, it could be stable at a wide range of pH. The  $K_m$  and  $V_{max}$  values of the enzyme were 0.825 mg.ml<sup>-1</sup> and 135.4  $\mu$ mol.min<sup>-1</sup>.mg respectively, using p-Nitrophenyl-beta-D-glucopyranoside as a substrate. The beta-glucosidase exhibited strongest hydrolysis effect on cellobiose and some its activity could be inhibited by SDS, Fe<sup>3+</sup> and Hg<sup>2+</sup>.

PMID: 11189362 [PubMed]

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**Fractionation of cellulase and beta-glucosidase in a *Trichoderma reesei* culture liquid by use of two-phase partitioning.****Brumbauer A, Johansson G, Reczey K.**

Department of Agricultural Chemical Technology, Budapest Technical University, Hungary.

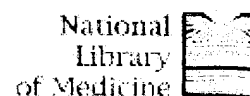
An aqueous two-phase system based on the two polymers poly(ethylene glycol) and dextran has been used for the fractionation of cellulase enzymes present in culture liquid obtained by fermentation with *Trichoderma reesei*. The activities of beta-glucosidase and glucanases were separated to high degree by using the two-phase systems for a counter-current distribution process in nine transfer steps. While the glucanases had high affinity to the poly(ethylene glycol) rich top phase the beta-glucosidase was enriched in the dextran-containing bottom phase. Multiple counter-current distribution performed indicates the heterogeneity of beta-glucosidase activities assuming at least four isoenzyme forms. One step concentration of beta-glucosidase by using system with 46:1 phase volume ratio resulted in 16 times higher enzyme activity.

PMID: 10643639 [PubMed]

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## Purification, characterization, and substrate specificity of a novel highly glucose-tolerant beta-glucosidase from *Aspergillus oryzae*

Riou C, Salmon JM, Vallier MJ, Gunata Z, Barre P.

Laboratoire de Microbiologie et Technologie des Fermentations, Institut National de la Recherche Agronomique, Institut des Produits de la Vigne, F-34060 Montpellier Cedex 2, France. riou@ensam.inra.fr

*Aspergillus oryzae* was found to secrete two distinct beta-glucosidases when was grown in liquid culture on various substrates. The major form had a molecular mass of 130 kDa and was highly inhibited by glucose. The minor form, which was induced most effectively on quercetin (3,3',4',5,7-pentahydroxyflavone)-rich medium, represented no more than 18% of total beta-glucosidase activity but exhibited a high tolerance to glucose inhibition. This highly glucose-tolerant beta-glucosidase (designated HGT-BG) was purified to homogeneity by ammonium sulfate precipitation, gel filtration, an anion-exchange chromatography. HGT-BG is a monomeric protein with an apparent molecular mass of 43 kDa and a pI of 4.2 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing polyacrylamide gel electrophoresis, respectively. Using p-nitrophenyl-beta-D-glucoside as the substrate, we found that the enzyme was optimally active at degreesC and pH 5.0 and had a specific activity of 1,066 micromol min<sup>-1</sup> mg protein<sup>-1</sup> and a Km of 0.55 mM under these conditions. The enzyme is particularly resistant to inhibition by glucose (Ki, 1.36 M) or glucono-delta-lactone (Ki, 12.5 mM), another powerful beta-glucosidase inhibitor present in wine. A comparison of the enzyme activities on various glycosidic substrates indicated that HGT-BG is a broad-specificity type of fungal beta-glucosidase exhibits exoglucanase activity and hydrolyzes (1-->3)- and (1-->6)-beta-glucosidic linkages most effectively. This enzyme was able to release flavor compounds, such as geraniol, nerol, and linalol, from the corresponding monoterpenyl-beta-D-glucosides in a grape must (pH 2.9, 90 g of glucose liter<sup>-1</sup>). Other flavor precursors (benzyl- and 2-phenylethyl-beta-D-glucosides) and prunin (4',5,7-trihydroxyflavanone-7-glucoside), which contribute to the bitterness of citrus juices, are also substrates of the enzyme. Thus, this novel beta-glucosidase is of great potential interest in wine and fruit juice processing.